

IN THE CLAIMS

Please cancel claim 32.

1. (presently amended) A method for purifying a ligand-binding molecule from a mixture, comprising:

(a) ~~forming a carrier-ligand conjugate by intein-mediated ligation wherein one of a carrier or a ligand is fused to an intein and the intein is cleaved resulting in a C-terminal thioester, the carrier reacting with the ligand to form the conjugate by means of a thioester-nucleophile reaction by reacting a C-terminal thioester on a carrier, with a nucleophilic group on a ligand or a C-terminal thioester on a ligand with a nucleophilic group on a carrier, wherein cleaving a carrier-intein fusion protein or a ligand-intein fusion protein generates the C-terminal thioester;~~

(b) binding the carrier-ligand conjugate to a matrix and contacting the carrier-ligand conjugate with a mixture containing the ligand-binding molecule to selectively bind the ligand-binding molecule to the carrier-ligand conjugate; and

~~(c) selectively binding the ligand-binding molecule of step (b) to the carrier-ligand conjugate; and~~

(c) (d) eluting the ligand-binding molecule from the ligand so as to obtain the purified ligand-binding molecule.

2. (original) A method according to claim 1, wherein the ligand-binding molecule is an antibody and the mixture is an antiserum.

3. (original) A method according to claim 1, wherein the carrier is a matrix-binding molecule.
4. (original) A method according to claim 1, wherein the matrix-binding molecule is selected from the group consisting of: a monosaccharide-binding-domain, a disaccharide-binding domain, an oligosaccharide-binding domain, a chitin-binding domain, a maltose-binding domain, an arabinose-binding domain, an arabinogalactan-binding domain, a lectin-binding domain, a vitamin binding-domain, a nucleic acid-binding domain, an amino acid-binding domain, a metal-binding domain, a receptor-binding domain, a sulfate-binding domain and a phosphate-binding domain.
5. (previously presented) A method according to claim 1, wherein the matrix-binding molecule is a carbohydrate-binding molecule.
6. (previously presented) A method according to claim 5, wherein the carbohydrate-binding molecule is chitin-binding domain.
7. (previously presented) A method according to claim 1, wherein the matrix is chitin, the matrix-binding molecule is a chitin-binding domain, the ligand is an antigen and the ligand-binding molecule is an antibody.
8. (original) A method according to claim 1, wherein the ligand is selected from an antigen, an antibody, a receptor, a receptor-binding domain, an enzyme and an enzyme substrate.

9. (withdrawn) A method for forming an affinity matrix for binding a ligand-binding molecule, comprising:

(a) forming a C-terminal thioester by cleavage of a fusion protein wherein the fusion protein comprises a carrier fused to an intein or a ligand fused to intein, such that cleavage occurs in the presence of a thiol reagent at the intein junction with the carrier or the ligand;

(b) combining in a mixture, either (i) the carrier with the C-terminal thioester and the ligand with an N-terminal cysteine or selenocysteine or (ii) the carrier with an N-terminal cysteine or selenocysteine and the ligand with the C-terminal thioester; and

(c) permitting the carrier to bind the matrix to form with the ligand after ligation, the affinity matrix.

10. (withdrawn) A method according to claim 9, wherein the carrier is selected from the group consisting of: a monosaccharide-binding domain, a disaccharide-binding domain, an oligosaccharide-binding domain, a chitin-binding domain, a maltose-binding domain, an arabinose-binding domain, an arabinogalactan-binding domain, a lectin-binding domain, a vitamin binding-domain, a nucleic acid-binding domain, an amino acid-binding domain, a metal-binding domain, a receptor-binding domain, a sulfate-binding domain and a phosphate-binding domain.

11. (withdrawn) A method according to claim 9, wherein the carrier is selected from M.HhaI, paramyosin, CBD and MBP.

12. (withdrawn) A ligand-binding molecule affinity matrix comprising:

a carrier conjugated to a ligand by means of a thioester-nucleophilic interaction; wherein one of the carrier or ligand has a nucleophilic group and the other has a reactive thioester group, the carrier-ligand conjugate being immobilized on a matrix, and the ligand in the carrier-ligand being optionally capable of reversibly binding a ligand-binding molecule.

13. (withdrawn) A ligand-binding affinity matrix according to claim 12, wherein if the carrier-ligand is immobilized non-specifically, the carrier is selected from M.HhaI, Paramyosin, CBD and MBP.

14. (withdrawn) A method for screening for the interaction of one or more immobilized ligands with one or more ligand-binding proteins in a preparation comprising:

- (a) covalently linking a carrier to a ligand by means of thioester-nucleophilic interaction to form a carrier-ligand conjugate;
- (b) permitting the carrier-ligand to be immobilized by a matrix;
- (c) reacting a preparation containing one or more ligand-binding proteins to the carrier-ligand; and
- (d) detecting the binding of the one or more ligand-binding protein with the one or more immobilized ligands.

15. (withdrawn) A method according to claim 14, wherein the carrier protein is M.Hha.

16. (withdrawn) A method according to claim 14, wherein the carrier protein is paramyosin.
17. (withdrawn) A method according to claim 14, wherein the carrier protein is selected from chitin-binding domain and maltose-binding protein.
18. (withdrawn) A method according to claim 14, wherein the preparation contains one or a mixture of proteins.
19. (withdrawn) A method according to claim 14, wherein the preparation contains an affinity purified antibody.
20. (withdrawn) A method according to claim 14, wherein the matrix is selected from nitrocellulose and nylon.
21. (withdrawn) A method according to claim 14, wherein the matrix is an SDS-polyacrylamide gel.
22. (withdrawn) A method according to claim 14, wherein the matrix is a synthetic polymer.
23. (withdrawn) A method according to claim 22, wherein the synthetic polymer is a polystyrene micro-titer plate.
24. (withdrawn) A method according to claim 14, wherein the preparation contains a single ligand-binding molecule or a set of

ligand-binding molecules such that only one type of ligand-binding molecule in the mixture binds to the carrier-ligand.

25. (withdrawn) A method according to claim 14, wherein the carrier-ligand is one of a set of fusion proteins such that each fusion protein contains a different ligand fused to a carrier, the fusion proteins being located on the matrix in an ordered array for detecting interactions between ligand and ligand-binding molecules.

26. (withdrawn) A method according to claim 14, wherein the fusion protein has a combined molecular weight in greater than 5,000 Da.

27. (withdrawn) A method according to claim 14, wherein the ligand is a protein having one or more post-translational or chemical modifications such that the ligand-binding domain is specific for the modified protein.

28. (withdrawn) A method for enhancing the immunogenic properties of a ligand such as a peptide antigen in a animal, comprising:

(a) forming a carrier-ligand fusion protein by intein-mediated ligation; and

(b) administering an effective dose of the carrier-ligand fusion protein to the animal to obtain an enhanced immune response compared with the ligand in the absence of the carrier.

29. (withdrawn) A ligand-carrier protein fusion molecule, comprising: ligand fused to Hha methylase carrier protein.

30. (withdrawn) A ligand-carrier protein fusion molecule, comprising a ligand fused to paramyosin.

31. (withdrawn) A method for screening for carrier proteins with affinity to one or more matrices; comprising:

(a) linking each of a plurality of carriers to a ligand by means of a thioester-nucleophilic interaction to form a plurality of carrier-ligands;

(b) permitting the carrier-ligand to bind to a matrix and to a labeled ligand-binding molecule that produces a signal when bound to a matrix; and

(c) comparing the signal on the matrix for the plurality of carriers to determine the affinity of the carriers for the matrix.

32. (cancelled)